REMARKS

Claims 1 and 3-18 are pending in the application. Claim 3 is amended to remedy an extraneous "the". Applicants respectfully submit that no new matter has been added.

1. Rejections under 35 U.S.C. §103

The Examiner rejects claims 1, 3-4, 8-12, and 13-18 under 35 U.S.C. § 103 as being unpatentable over Tropepe, Weiss, and Suemori. The Examiner also rejects claims 5-7 under 35 U.S.C. § 103 as being unpatentable over Tropepe, and Weiss, in view of Vitokovic, Reubinoff, and Thompson.

A. Tropepe, Weiss and Suemori

a. Tropepe discloses a neurosphere, not a stem cell sphere as claimed, and the methods are fundamentally different.

The Specification discloses that methods of making a neurosphere are known within the art. (Specification, page 2, line 17). However the presently claimed methods generate stem cell spheres. The differences between the two are disclosed in the Specification at page 2, line 2 to page 3, line 8. In particular, embryonic neurospheres are cultured with a defined medium, and while it has self-renewal capabilities, the ultimate population of differentiated neural cells arises from a small portion of the total population of neural stem cells. In contrast, the present invention requires culturing in an undefined medium (astrocyte conditioned medium), where the cells of the stem cell sphere migrate from the SCS as neural stem cells in large quantities.

The disclosure of Tropepe suggests that the cells it produced were embryonic neurospheres. Regarding the Examiner's statements on page 3, lines 1-6 of the Office Action dated December 10, 2009, Tropepe describes that the dispersed ES cells are cultured at a low cell density (20 cells/pl), thereby forming colonies derived from single ES cells at a frequency of about 0.2% in the presence of LIF alone (page 66, second column, line 5 of second paragraph, and Fig. 1-A of Tropepe). Also, Tropepe clarifies that when subcultured, the colony-forming ES cells have a colony forming rate of the same level as that of the primary subclones of embryonic bFGF-

responsive neural stem cells (page 67, first column, 14 to 10 lines from bottom of Tropepe). In addition, Tropepe describes in the section of Discussion on page 73, first column, 2 lines from bottom that "neural colonies generated from ES cells share some similar features to forebrain stem cell colonies"; therefore, the colonies from the ES cells are considered to share some similar features to those of the embryonic neural stem cell colonies. Further, Tropepe continues to describe in the same paragraph from first column, bottom line to through second column, 6 lines to bottom that particularly the mechanism for separation of the subpopulation of neural stem cells capable of colony-forming from large subpopulation of neural cells may be attributed to neural fate specification from the ES cells. In view of the above, the colony derived from single ES cells is equivalent to embryonic neurospheres (Tropepe et al, *Dev. Biol.* 208: 166-88, 1999)(attached). Therefore, one of ordinary skill in the art cannot expect the preparation of the stem cell sphere taught in the present invention, which is completely different in the nature and the structure from those disclosed in Tropepe.

Further, Tropepe clarifies that the ES cells are differentiated into Nestin+, β III-tubulin+, and NeuN+ cells in the absence of an exogenous factor within 24 hours (page 71, the entire section of "Neural Cell Fate Is Rapidly Established from ES Cells in the Absence of Exogenous Factors"). This description suggests a possibility that the ES cells autonomously differentiate into neural cells (page 74, second column, the section of "Default Neural Cell Fate Specification during Mammalian Development," 4 to 9 lines from top of the first paragraph of this section). One of ordinary skill in the art cannot expect a method of differentiating neural stem cells and neural cells using a liquid factor contained in ACM from the experimental results of Tropepe and interpretations drawn therefrom.

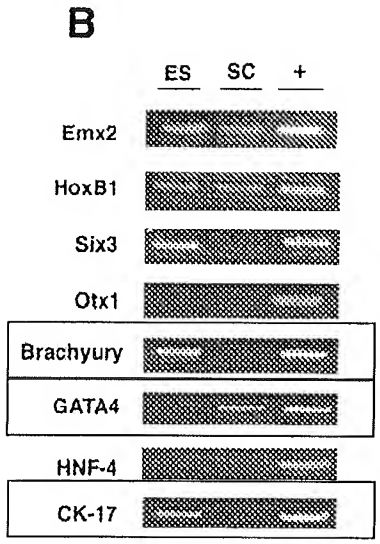
Moreover, Tropepe concludes that the LIF upon formation of the colony acts as a permissive factor to maintain the ES cells in an undifferentiated state (pages 68-69, the whole section of "LIF Functions as a Permissive Factor for Neural Stem Cell Differentiation of ES Cells"; especially, the sentence that reads: "Thus, LIF is critical ... in these minimal conditions" on page 69, first column, lines 9-12 of Tropepe). Furthermore, Tropepe clarifies that the colony

formation is carried out in the presence of LIF by reacting the ES cells themselves to endogenous FGF-2 secreted by the ES cells (page 68, first column, 3 lines from bottom to page 69, first column, line 5).

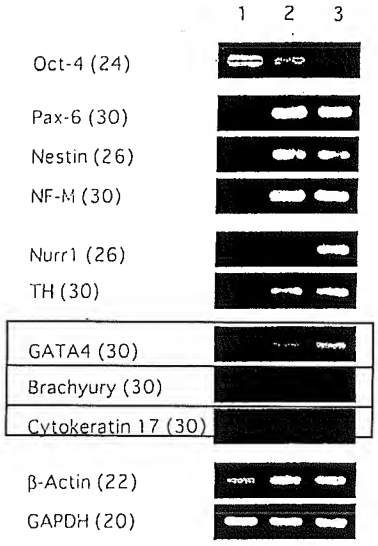
But, in the present invention, LIF is not added to ACM exogenously. In addition, in the SCS method of the present invention, FGF-2 (bFGF) acts to proliferate the neural stem cells. Therefore, in the present invention, the differentiation of the neural stem cells is carried out under a completely different technical idea from that of Tropepe.

Tropepe only discloses a method for proliferating neural stem cells by preparing a colony equivalent to a neurosphere from relatively small numbers of ES cells. One of ordinary skill in the art could not expect the differentiation of a colony of undifferentiated ES cells directly into neural stem cells or neural cells as taught in the present invention from the teachings of Tropepe.

Also in the ES-derived spheres of Tropepe, the gene expression of GATA4, brachyury, and CK-17 is different from the cells obtained in the present method. Compare (Fig. 2-B) of Tropepe with Figure 23 of the present method. In Figure 2B of Tropepe, the ES (primary ES cells), SC (ES-derived sphere colonies, and + (positive tissue control) might be compared to Figure 23 column 1 (undifferentiatied embryonic stem cells, Specification, page 5, line 23), column 2 (the SCS formed by carrying out the suspension culture, *Id.*) and column 3 (the cell masses obtained by carrying out the adhesion culture). It is clear that the brachyury and CK-17 expression is significantly different between the cell populations, and that the GATA4 expression is likely different as well.



Tropepe Figure 2



Specification Figure 23

It is evident that the spheres of Tropepe have properties different from the SCS in the present invention in which the expression level of the gene of GATA4 does not increase.

Other characteristics between the cells are different as well. Tropepe clarifies that colony formation from the ES cells and the differentiation of the neural stem cells is suppressed by BMP4 of the TGF9 family (page 69, second column, the section of "Inhibition of TGFI3-Related Signaling Enhances Neural Stem Cell Differentiation of ES Cells, Figure 5.). However, in the presently claimed method method of differentiation is not suppressed in BMP4 (Nakayama et al. *Neurosci Res* ***46:*** 241-249, 2003, page 244, second column, last paragraph)(attached).

Furthermore, the presently claimed method obtains a single cell type. The method of Tropepe obtains a colony of cells which includes three different cell types. (Tropepe, page 67, second column, line 5). The differentiation of a neurosphere is characteristically into a heterogeneous cell type. Thus, once again, the presently claimed method is fundamentally different from that disclosed in Tropepe.

Accordingly, Applicants submit that the method of Tropepe is fundamentally different from that of the claimed invention. Furthermore, one of skill in the art would have no reason to expect the cell population obtained with the claimed method by combining the methods of Tropepe and the other references because the basic method of Tropepe is fundamentally different.

b. Weiss does not change the fundamental method disclosed in Tropepe.

Nothing in Weiss suggests that the combination of the methods in Weiss and Tropepe would change the Tropepe method into a fundamentally different method to obtain a different population of cells. First, like Tropepe, Weiss also discloses the neurosphere method. Second, Weiss discloses a completely different origin of the cells, such that one of skill would not apply the teachings of Weiss to Tropepe. Third, Weiss does not disclose an astrocyte conditioned medium or a medium equivalent to ACM. Fourth, Weiss teaches away from the omission of FGF.

As a preliminary matter, Applicants submit that Weiss is a development group for the neurosphere method discussed in the Specification, beginning at page 2, line 14 (Reynolds and Weiss, *Science* 255: 1707, 1992, Reynolds et *al., J. Neurosci* 12: 4565, 1992, which refer to the method of column 12, lines 29-31 of Weiss)(both attached), and the method of Weiss is carried out using the medium of the Reynolds neurosphere method (column 8, last paragraph of Weiss).

For instance, although the heading of Example 3 on column 12 of Weiss is Embryonic Stem Cells, the content is on neural stem cells, the samples of Weiss are collected from the brain and striata of E14 mice (Example 3: column 12, lines 11-12), not from blastocyst inner cell mass. Thus, the initial cell type of Weiss is different from both Tropepe and the claimed method. Applicants submit that one of skill would not expect that the method of Weiss would be applicable in the same manner to that of Tropepe.

Also, the medium used in the formation of the neurosphere of Weiss (column 12, lines 17-25 and FIG 2) is a "Complete Medium" (column 6, 2 lines from bottom to column 7, line 7), a Defined medium, supplemented with EGF (column 12, line 16 and 18), not a conditioned medium (CM). Like the Reynolds papers discussed above, the method of Weiss is carried out using the medium of the Reynolds neurosphere method (column 8, last paragraph of Weiss).

Applicants submit that the method of Weiss does not prepare neurons in the absence of bFGF. (Office Action, page 3, line 20 to page 4, line 1). First, the cells that are prepared in Example 3 of Weiss are somatic neural stem cells, not neural stem cells prepared from embryonic stem cells. In addition, the conditions in which TH^+ exist in the neurosphere prepared in Example 3 is a conditioned medium (BCM) + FGF-2 (column 15, line 9), "in the absence of bFGF" as suggested by the Examiner.

Furthermore Weiss *teaches away* from the present method of obtaining neurons as in claim 12, which excludes bFGF, by suggesting that BCM and FGF-2 are required. Weiss confirms that neural stem cells from an adult subventricular zone are differentiated into TH^+ neurons, so that it is thought that a possibility of preparation of TH^+ neurons not only from fetal tissues but also from adult brain is taken into consideration with autografts from patients with a Parkinson's disease in mind (column 3, lines 44-48). However, this is the differentiation from adult neural stem cells in the presence of BCM and FGF-2.

This teaching away is supported by the disclosure of Examples 6 and 8 in Weiss. It is evident from Example 6 of Weiss that in Paradigm 1 in which ability of ACM and BCM to induce differentiation into TH^+ neurons is evaluated using an embryonic cortical primary culture, and Paradigm 2 in which the ability on an astrocyte feeder layer is evaluated, the differentiation does not take place unless FGF is present (columns 13-14, TABLES I and II). Also, in Example 8 where similar effects are evaluated with the neurosphere prepared in Example 3, the necessity of FGF-2 is confirmed in Weiss. Therefore, the present invention teaching the differentiation into neural stem cells or neural cells from ES cells in the presence of ACM alone could not be expected from the teachings of Weiss.

Thus, Applicants submit that one of skill in the art, regarding the teachings of Tropepe and Weiss would have no reason to omit bFGF.

c. The addition of Suemori does not remedy the deficiencies of Tropepe and Weiss.

Suemori is cited for the disclosure of methods of monkey embryonic stem cell culture. Applicants note that the citation referred to by the Examiner is merely a discussion in Suemori citing another paper, by Thomson et al., *Science* 282:1145-7, 1998., 1145, second column, lines 12-15.

The Suemori publication focuses on the fact that ES cells of monkey can be maintained in an undifferentiated state for a long period of time, and then differentiated into various types of tissues.

The differentiation of ES cells in Suemori is by an embryoid body method (page 275, first column, Figure 2). Therefore, Applicants submit that the content of Suemori is not relevant to the presently claimed method. Furthermore, any suggestions of applying the method of Suemori to human ES cell therapies are again not relevant to the claimed method.

Furthermore, the cryopreservation in Suemori is of a different cell type. Specifically, Suemori discusses that cynomologous **ES cells** can be cryopreserved (page 274, second column, 2 lines from bottom to page 275, first column, line 1). This is different from claim 12 of the present application where **neural stem cells** are cryopreserved. The two are at a different stage of differentiation. Accordingly, Applicants submit that at the very least, Suemori does not render obvious at the very least.

d. In summary the rejection based on Tropepe, Weiss, and Suemori should be withdrawn.

The Examiner's rejection of claims 1, 3-4, 8-12, and 13-18 as being unpatentable over Tropepe, Weiss, and Suemori should be reconsidered and withdrawn because the method of Tropepe refers to a fundamentally different method to obtain a completely different cell type. Furthermore, Weiss does not provide any suggestion to change the method such that it would obtain the same cell type. Weiss also teaches away from the present invention. Likewise, Suemori discloses the embryoid body method and teaches that a different cell type can be cryopreserved. None of these teachings would inform one of skill in the art on how to perform the method of the claimed invention, or give them any expectation of success in achieving the homogenous cell population produced by the claimed method in a reasonable yield.

B. Tropepe, Weiss, Vitkovic, Reubinoff, and Thompson

The deficiencies of Tropepe and Weiss are discussed above. However, the combination of Vitkovic, Reubinoff, and Thompson does not remedy these deficiencies.

a. The ACM of the present inventive method is different from that used in Vitkovic.

Applicants submit that the ACM used in Vitkovic is different from the ACM used in the present method, such that the combination of this medium with the methods of Tropepe and Weiss is not an ACM or a medium equivalent thereto. Specifically, the ACM used in Vitkovic contains sera, and with or without a reactive component obtained by lipopolysaccaride stimulation.

The astrocyte-conditioned medium used in Vitkovic is prepared according to a publication made reference therein (Ref. 12: Vitkovic et al., *J. Neuroimmunol* 30: 153-60, 1990)(attached). The Vitkovic 1990 publication describes that astrocytes are cultured in a

serum-containing medium (page 154, second column, the section of "Material and method, Culture media," lines 7-10). Further, astrocytes are stimulated by adding a lipopolysaccaride (page 155, first column, the section of "Preparation of astrocyte-conditioned supernatant," lines 1-8). Therefore, the ACM used in Vitkovic contains sera, and with or without a reactive component obtained by lipopolysaccaride stimulation.

On the other hand, the ACM used in the present invention is prepared based on a serum-free, defined medium and DMEM/F12/1% N2 supplement (Nakayama and Inoue, *Methods in Molecular Biology* 330: 1-13, 2006: page 3, 2.1.1. Media 3.; page 6, 3.1.5.2. Production of Astrocyte-Conditioned Medium)(attached), in which the influence of the sera to the ES cells is removed.

Accordingly, one of skill in the art would have no reasonable expectation of success in combining the fundamentally different methods of Tropepe and Weiss with the significantly different medium of Vitkovic to obtain the cell population obtained by the claimed method. Applicants request that the rejection be withdrawn.

b. Like Weiss, Reubinoff teaches away from the omission of bFGF.

Applicants submit that Reubinoff teaches away from the presently claimed method and would not give one of skill in the art any reasonable expectation of obtaining a neuron in the absence of bFGF. Reubinoff describes that in order to commit the differentiation of the spheres from the generated ES cells to glial lineage, the medium is supplemented with PDGF-AA, bFGF, and EGF, followed by culture in the presence of T3 (page 1139, second column, lines 22-27). Also, Reubinoff points out the importance of bFGF and PDGF-AA in the enhancement of the proliferation of the glial precursor (page 1136, second column, lines 34-41, quoted ref. 14: Brustle et al., Science 285: 754-756, 1999: page 754, second column, lines 4-7).

The differentiation into astrocytes as described in Reubinoff is carried out according to a conventional method using bFGF and PDGF-AA. Therefore, it would not be obvious for one of

ordinary skill in the art to expect the present invention in which the spheres are differentiated into astrocytes in a Defined medium (Neurobasal/B27 supplement) alone in the absence of bFGF from the method of Reubinoff.

c. One of skill would not have any reasonable expectation of success based on the general speculation in Thompson.

Applicants submit that the citations relied on by the Examiner from Thompson are merely speculation by the authors and would not provide any reasonable expectation of success to one of skill in the art. Specifically, the description on the paragraph bridging pages 1146 and 1147 of Thompson is merely mentioning an expectation as a matter of course that specialized cells can probably be generated from the ES cells once the mechanism of the differentiation from the ES cells is elucidated. Also, the applications of the generated cells from human ES cells, the development of drugs and the applications to the transplantation therapies and the like are *merely* general descriptions. Accordingly, Applicants submit that one of skill in the art would not find the present inventive method obvious from the general speculation in Thompson.

In conclusion Applicants submit that one of skill in the art would not have found the claimed method obvious based on Tropepe, and Weiss, in view of Vitokovic, Reubinoff, and Thompson for the reasons stated above with Tropepe and Weiss and because the media of Vitkovic is different from that of the claimed method, Rebinoff requires FGF and Thompson merely speculates but provides no guidance for obtaining a neural cell population. Applicants respectfully request that the rejection be reconsidered and withdrawn.

In view of the foregoing, Applicants believe the pending application is in condition for allowance. A Notice of Allowance is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mary M.H. Eliason, Reg. No. 58,303 at (858) 792-8855, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

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Attachments- Tropepe et al, Dev. Biol. 208: 166-88, 1999
Nakayama et al. Neurosci Res 46: 241-249, 2003
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